

# *The Ohio Naturalist,*

PUBLISHED BY

*The Biological Club of the Ohio State University.*

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Volume XIII.

JUNE, 1913.

No. 8.

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## THE MOSAIC DISEASE OF THE TOMATO AND RELATED PLANTS.\*

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### INTRODUCTION AND HISTORICAL SUMMARY.

The mosaic disease or calico of Solanaceous plants seems to be one of those pathological problems, which has resisted the efforts of the scientist and baffled the most observant layman for the last half century. That progress has been made in the study of mosaic disease is obvious, but the great problem of its cause still remains to be solved. In the review of its literature, it will be noticed that contradictory and conflicting results and conclusions have been so numerous, in the scientific investigations of this problem, that one cannot accept the results unconditionally. In order to summarize the results, conclusions and theories of past investigators, and to make the literature pertaining to this disease more accessible, the writer has endeavored to present a review and bibliography of the essential literature of mosaic disease. It is hoped that this will provide a reliable basis for future work.

The first reference to the disease according to Hunger (1905, p. 256), was by Swieten (1857), who mentions a disease which resembles the mosaic disease of tobacco. This disease was called "Rost" or Fleckenkrankheit (Spot disease), terms by which mosaic disease was known for some time. In 1885, Adolf Mayer investigated this disease on tobacco and in the following year published an account of it, naming it "Mosaic Disease." Koning (1899, p. 65), states that Dr. van Breda de Haan, called his

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\*Contributions from the Botanical Laboratory of Ohio State University. No. 74.

attention to this tobacco trouble, stating that it had occurred in the East Indies in 1888. The next investigator of this problem whose work attracted attention, was Iwanowski (1892, 1899, 1903), who most emphatically pronounced mosaic disease to be bacterial in nature. Prillieux and Delacroix (1894), describe the disease, believing that it is similar in nature to a spot disease occurring on *Cyclamen*. Marchal (1897), mentions mosaic disease and its treatment. Koning (1897), describes specific organisms which are supposed to be associated with this disease. Beyrerinck (1898), and Sturgis (1899), both published papers. The former author propounded the "contagium vivium fluidum" theory, while Sturgis regarded it as a physiological trouble. The following year (1900), Sturgis published the results of experiments in shading and liming tobacco plants. Woods (1899) presented his paper on the destruction of chlorophyll by oxidizing enzymes, with special reference to mosaic disease. According to Hunger (1905, p. 262), Dr. van Breda de Haan (1899), isolated bacteria from the tissues of diseased plants, said to be affected with mosaic. In (1900) Heintzel published a paper on tobacco mosaic and Behrens mentioned a disease of the tobacco which resembled mosaic in its symptoms and characteristics. Gontiere (1900), in a short review gives recommendations for treating seed and seed-beds. Woods (1902) revolutionized the interpretations of this malady, by propounding his enzyme theory and Hunger (1902, 1904), believed that he had eliminated bacteria as the causal organism. But nevertheless in the following year, Hunger (1903) (a) severely criticised Woods' enzymic theory. Suzuki (1903) studied a peculiar variegation of the leaves of the mulberry, obtaining results similar to those of Woods' on tobacco. Hunger (1903) (b) published other work explaining some of the ways in which this disease is spread. In the same year Boyugues (1903), cites definite data, dealing with the incubation of mosaic disease; he also seems to have made an anatomical study of the trouble. That laborers are responsible for the spreading of this disease in part, is shown by Hunger (1903). Selby (1904) confirmed some of Hunger's infection experiments, showing that the disease could be disseminated by alternately touching diseased and healthy plants. In (1905) Hunger published a detailed treatise on mosaic disease, treating of its history, theories and experimental data. Delacroix (1905) found that a bacillus is associated with mosaic disease, and gave its exact measurements. Clinton (1908) mentions tomato chlorosis and its characteristics; he speaks of a similar malady on lima bean. Later (1910) he mentions as similar troubles, chlorosis of the squash, muskmelon and tobacco. Tomato mosaic is treated and compared with the same disease of tobacco by Westerdijk (1910). Loedwijks (1910) shows how colored light and light intensities effect the behavior of

diseased plants. Shaw (1910) believes the Curly Top of sugar beet to be a trouble pathologically and physiologically related to mosaic disease. Allard (1912) believes that Aphids are carriers of mosaic disease.

*Nomenclature.*—The names which have been applied to this singular disease, have been many and varied. In America, mosaic disease, calico, Frenching, mottle-top and chlorosis are terms applied in the Central States; while in the south, brindel or mongrel disease are more common. In Germany one hears of it as Mosaikkkrankheit, Mauche, Fleckenkrankheit or Pockenkrankheit; in France la Mosaïque, Nielle or Rouille blanche and in Hungary, Mozaik-betegsege. In Italy it is known as Mal del Mosaico or Maldella bolla and in southern Russia the name Bosuch seems to be the most used. Poetih is the name applied in Sumatra, Java and Borneo. Besides these names there are many colloquial expressions in use. Special names applied to Pockenkrankheit are: "Ospa" (Pox) in Russia; "Rjabucha" (Dot like), in Little Russia; "Pestrizi" (Spots) in S. W. Russia.

#### Hosts.

This disease, although originally described only on tobacco, has in recent years been found on numerous other hosts. Woods (1902) describes it as being produced artificially on the potatoes, Petunias, Violets and poke weed, and Iwanowski (1903) speaks of it as occurring on the beet and kidney bean. Similar troubles have also been found by Suzuki (1902) on the Mulberry, by Selby (1904) on cucumbers, by Clinton (1910) on lima and string beans, muskmelon and squash. Some investigators would place mosaic disease in the same class with albinism or variegation; (Woods 1899). Orton reported it on potatoes at the Cleveland meeting of the American Phytopathological Society, 1912-13, and the writer has recently found it occurring naturally on the potato in the greenhouse.

#### CHARACTERISTICS.

*Tobacco.*—As already indicated above, this malady seems to be present throughout the tobacco growing regions of the world, although there are some countries growing tobacco extensively from which no reports of its occurrence have been seen.

This disease usually makes its first appearance either in the seed-bed or cold-frame. The middle or lower leaves are the first attacked and gradually the uppermost leaves show the characteristic symptoms. The disease reveals itself on the leaves by an irregular, more or less mottled effect, a differentiation into yellowish and dark green areas. The dark green areas are often confined quite largely to a border along the larger veins, while the intermediate tissue assumes a lighter green or yellowish hue. Upon closer inspection differences may be noticed; the adjoining

green regions seem slightly swollen, while the yellow areas appear appreciably thinner. Many of the affected leaves become crinkled or show an irregular growth; this is due to an uneven tissue expansion; the healthy green regions develop more rapidly than the yellow areas, hence a warping or crinkling results. Woods (1902) states that in very severe cases the entire plant may become so deformed that it is almost unrecognizable.

As the plant becomes older and the flower buds form, there may appear what is known as "mottle top," although the plant may have remained perfectly healthy up till flowering time. According to Sturgis (1899), weather conditions may bring on the disease at this time and affected plants may recover if conditions become favorable again. He regards "mottle top" as a later stage and milder form of calico; the typical mosaic appearing only in the earlier stages of plant development. The writer has occasionally encountered this in the field and from his observation it does not seem serious, as it apparently involves only a few of the uppermost leaves, which are always removed at topping time.

*Tomato.*—Where tomatoes are forced under glass, mosaic disease is not uncommon and appearances similar to mosaic are also found in the field. One of the first investigators to call our attention to the mosaic disease on tomato was Sturgis (1899). He cites a case where a field of tomatoes was overtaken by an early frost and severely nipped. As a result of this artificial pruning, the disease made its appearance. Woods (1902) produced the disease at will on tomato and poke weed by severely pruning healthy plants. See his plates 2, 5 and 6. Tomato chlorosis and its infectious properties are discussed by Clinton (1908). Hunger (1905) seems to be the first foreign investigator who worked with tomato mosaic. He confirmed Woods' (1902) pruning results, having used plants grown from seed from various countries. Westerdijk (1910) carried out extensive experiments with tomato mosaic, which show the disease is inheritable. According to her the disease is conspicuous on stalks and fruit as well as leaves. She says that the stalks frequently show a spiral band of yellow color. During the earlier stages of fruit development, while it is still green she says, that the yellow spots are easily recognized, but as the fruit matures, the deep red masks them.

The yellow areas on the leaves, as for tobacco, seem confined more or less to the tissues between the main veins. The dark green regions nearby seem to assume a rather "over healthy" aspect. Here again, an unequal growth of tissues cause the leaf to warp or curl. In severe cases, descriptively termed, "fern leaf" appears. Here the main veins are considerably hypertrophied, while the intermediate tissues altogether fail of development, giving the leaf a very striking dissected appearance.

Westerdijk (1910, p. 7) states, "a great share of the blossoms perish before fertilization is effective; either the flowers blight or drop off." She also states that diseased plants bear less fruit than normal and that the fruit which does set is usually small or malformed. This would naturally be expected where there is an apparent lack of proper nutrition, brought about perhaps by a reduction in the assimilative and digestive powers of the leaves.

It often happens that some of the lower leaves of tomato plants show yellow spots or are entirely yellow; this in most cases is due to improper light or soil conditions and should not be mistaken for mosaic disease.

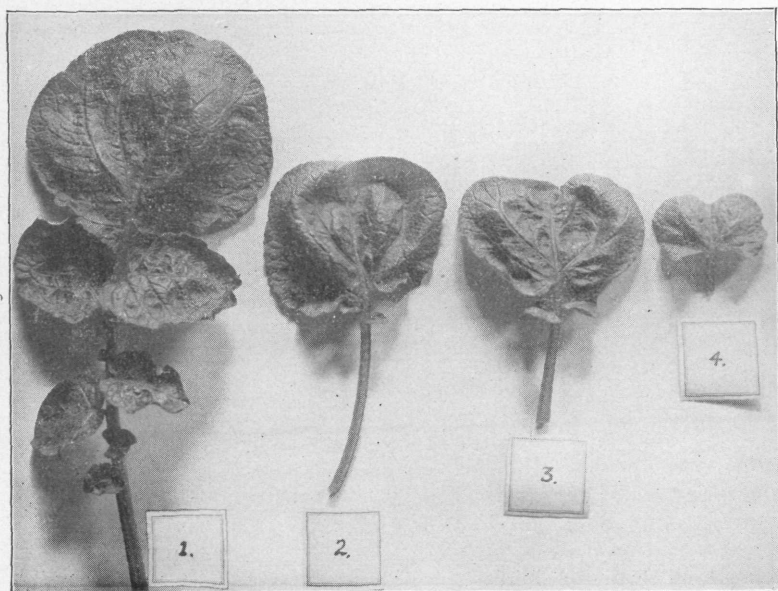


FIG. 1. Leaves from various parts of mosaic-diseased potato plants, showing surface irregularities, due to variable tissue expansion. Two-thirds natural size.

Where tomatoes are grown under glass, the extent of damage caused by this disease may vary from the injuring of a few scattered plants to the loss of a considerable share of the crop. In Ohio mosaic disease frequently appears in one or more of the main crops.

*Potato.*—During the month of February, 1913, mosaic disease appeared very suddenly in the Ohio State University greenhouse, on Early Lunch potatoes, which had been planted in sand for

the purpose of growing plants for breeding experiments. The writer has found no extensive description of mosaic disease on the potato in the literature, but it was reported by Orton (1913) as occurring in Germany and Maine.

The first symptoms were noticed on a plant which had reached a height of approximately eighteen inches. When first observed the plant appeared thrifty in every respect, except that the immature leaves had a slightly pale and mottled appearance. Four days later the yellowish spots were more pronounced and appeared on about two-thirds of the leaflets. The very youngest leaves were also conspicuously pale, with a sickly yellow color. In this early stage the mottled effect is not perceptible, but it becomes noticeable as the leaflets age. Those having practically reached their full development, occasionally showed a slight abnormality in shape or an uneven surface. See text Fig. 1. The mottled effect consists of irregular, greenish-yellow or pale yellow spots, which appear at any place on the leaf. See plate VII. As in tobacco and tomato mosaic, the yellow spots are localized in the tissue between the veins, which have a conspicuous border of dark green tissue. If such leaflets are sprinkled or submerged in water, the color differentiation is greatly intensified. In the majority of leaflets the green areas developed more rapidly than the yellow as usual in this disease. Such differences in growth cause a somewhat irregular surface.

Upon examination, it is to be observed that the hairs on the upper surface of the leaf are much closer together in the yellow areas than in the normal or in the green areas. It appears that the hairs develop as usual while the leaf is very young, but that there is less than the normal expansion of the leaf surface between them, so that they are left standing close together, giving the leaf a striking and peculiar appearance. The surface of a calicoed leaflet when examined under a hand lens, shows that the dark green areas are somewhat elevated, while the yellow areas are slightly depressed, giving the surface an uneven appearance. No peculiarities could be seen upon the stalks or petioles and hypertrophies were lacking. The disease appeared spontaneously without pruning or other mutilation or artificial stimulation, which is said to be sometimes responsible for the production of such deformities in the potato (Woods 1902), as well as in other hosts. The writer has not observed this trouble on potatoes growing in the field, but intends conducting experiments later. It might be stated that the tubers which produced these diseased plants came from New York.

## HISTOLOGY.

Koning (1899 [b], 1900) made histological studies of mosaiced leaves, but says that little is brought to light by microscopic examinations. Intercellular cavities occur between the palisade and spongy parenchyma of young and old tissue. In some cases he found the chloroplasts disorganized and cell walls disappearing. Bouygues (1903) reported the absence of the epidermis. In old spots the cell contents had disappeared. Woods (1900, p. 17) found that, "a study of the histology of the diseased leaves has now revealed a histological difference which makes it very clear that the light colored areas are not normal and that this difference consists in the fact that in badly diseased plants the palisade parenchyma of the light colored areas is not developed at all. All of the tissue between the upper and lower epidermis consists of a spongy or respiratory parenchyma rather more closely packed than normal. In moderately diseased plants the palisade parenchyma of the light area is greatly modified. Normally the palisade parenchyma cells of a healthy plant are from four to six times as long as broad. In a moderately diseased plant, however, the cells are nearly as broad as they are long, or at most not more than twice as long as broad. As a rule the modified cells of the leaf pass abruptly into the normal cells of the green area." He also found that the light colored areas in both tomato and tobacco contained more than the normal amount of starch. Heintzel (1900), does not mention any peculiarities in the palisade cells themselves, but observed the most striking differences in the intercellular spaces between the palisade cells and the spongy parenchyma of younger and older tissue. These intercellular spaces occur in the dark green, bloated regions, the older tissue having the larger spaces. He believed these spaces were filled with gas, because their dark color disappeared when they were put in alcohol. The chloroplasts were congregated irregularly in small groups. Iwanowski (1903) states that the green areas bordering the yellow are 'abnormally healthy' and that such regions show a vigorous development of all cellular tissue. The yellow areas on the other hand, are thinner and the palisade cells are not so well developed, being very much shortened and cuboidal in form. He speaks of intercellular spaces in the yellow areas. The chloroplasts in these areas are yellowish and while these regions are young, scarcely react to the starch test, but eventually all the chloroplasts come to contain as much starch as they can hold.

*Tomato.*—Westerdijk (1910) says that a microscopic examination of mosaiced tomato leaves show nothing worthy of mention. In the yellow areas the chloroplasts are yellowish and slightly smaller and have but little starch. The writer also made his-

tological studies of mosaiced tomato, but did not find any characteristic abnormalities. No striking differentiation was seen between the yellow and adjoining green or healthy tissues. I did not find stages as described by Woods (1900), where the palisade parenchyma was undeveloped or the presence of conspicuous cuboidal palisade cells as described by Iwanowski (1903) for tobacco. Although at times in the yellow areas this tissue appeared slightly less developed than usual. The yellow areas were slightly thinner than the adjacent green areas, especially in older leaves. The epidermis appeared normal. No difference was detected in the number or size of the chloroplasts in the yellow and green areas. That they were well supplied with starch was apparent from the slides and especially in the sections from the older tissue.

*Potato*.—Sections of yellow, adjoining green and healthy tissue of potato mosaic, were fixed in weak chromacetic fluid and imbedded in the usual manner. A microscopic study showed that the yellow areas were thinner at all ages; in some cases they were only 90 mic. thick as compared with 120 mic. in the normal leaf. (See Fig. 1, 2, pl. VIII.) This thinness was largely due to a shortening of the palisade cells which were of a striking cuboidal form (Fig. 1, pl. VIII). Sections from mottled areas were easily distinguished by the shape and size of the palisade cells. The cuboidal cells began very abruptly in some sections, while in other cases there was an intergradation between them and the normal palisade cells. In the yellow areas as a rule, these cells were generally quite regular in shape, but sometimes there was less regularity. Their length varied from one-half to one-third that of normal cells and their thickness was usually slightly greater. The spongy parenchyma appeared normal in all areas, except that in the yellow regions, there were somewhat fewer chloroplasts. Figure 3, pl. VIII, represents a green area, adjoining a yellow spot. The palisade cells are slightly shorter than in Fig. 2. The chloroplasts throughout the yellow regions in living material were a pale yellowish-green, but contained considerable starch.

#### CHARACTERISTICS OF MOSAIC DISEASE.

*Infectious*.—Investigators who have conducted inoculation experiments with this disease on tobacco find it transmissible by means of the juice. Mayer (1886), Sturgis (1899), Hunger (1905) and others, have shown that it must be classed as infectious rather than contagious, for the mere presence of a diseased plant in a healthy plot does not cause the disease to spread. Numerous investigators have inserted diseased leaf tissue into healthy plants and produced the disease; in grafting healthy and diseased plants, similar results were obtained, Iwanowski (1903), Woods (1902) and Hunger (1904, 1905). Heintzel (1900) states, that he got



positive results by inoculating with healthy as well as diseased tissue. The same results were obtained by Woods (1899, 1902). When an excess of virus is used, this disease on tobacco according to Beyerinck (1898), develops hypertrophies. Heintzel (1902) finds that the injection of small quantities of fluid from a diseased plant produced the mottled effect, while a large amount produced hypertrophies.

*Disease Spread by Contact.*—Some experimenters have transmitted this disease under field conditions by touching alternately diseased and healthy tobacco plants. Koning (1899) believes that mosaic disease is spread in the field by handling plants. Hunger (1903, 1904, 1905, p. 286), in his 'touching experiments' was successful in spreading this disease and "he believes that much of the disease as it appears is due to negligence on the part of the laborers in the field." Selby (1904), as stated above, confirmed Hunger's experiments, producing the disease in the same manner by touching. Hinson and Jenkins (1910) also believe that the disease may be spread in this manner.

*Spontaneous Occurrence.*—Sturgis (1900) comments on the sporadic nature of this disease and states that it is not uncommon to find healthy and diseased plants growing in the same spot. Woods (1902, p. 18) says, "of the remaining twenty-five controls, four were affected with the disease without apparent cause." Iwanowski (1903), could not account for the appearance of disease in plants which had in no way been treated, 'they simply appeared spontaneously.' Hunger (1904), likewise could not account for these sudden appearances where plants had not been touched; furthermore the disease did not always appear where diseased and healthy tobacco plants were alternately touched. Westerdijk (1910), speaks of it as reoccurring periodically after it has once appeared in a greenhouse where tomatoes have been grown, although a new strain of seed was used each season.

*Producing the Disease at Will.*—Woods' (1902) experiments show this disease may be produced at will, by pruning, mechanically injuring the plant in various ways or even by injecting distilled water! Hunger (1905), confirmed Woods' pruning experiments with tomatoes of various sorts, including red and yellow, rough and smooth fruiting varieties. He failed, however, to duplicate Woods' results in tobacco. Allard (1912), says that a true infectious mosaic disease cannot be produced by pruning plants.

*Cross Inoculation.*—It is not possible to transfer this disease from the tobacco to the tomato or vice versa, according to Westerdijk (1910, p. 18-19). "It is not inconceivable that the virus of the tobacco ought to be transmissible to the tomato and inversely, because the plants are closely related. This, however, is not the case. Numbers of tomato plants were inoculated

under the most favorable growing conditions, with the virus from tobacco plants. The inoculations had no effect. The virus from the tomato had just as little effect upon the tobacco plant. The tomato plants withstood the injections very nicely and did not show the least signs of distortion." Clinton (1908) on the other hand states that he succeeded in producing mosaic disease on the tomato by inoculation with juice from a diseased tobacco plant, and from this tomato plant he transferred the disease back again to tobacco.

*Is Mosaic Disease Inheritable?*—Investigators are almost unanimous in the opinion that "calico" of tobacco is not inheritable. Woods (1902, p. 7) says, "There is no conclusive evidence that the plants from seed of diseased plants are more subject to the disease than are those from the seed of healthy plants." Iwanowski (1903) conducted inoculation experiments with crushed diseased seed. He produced the disease in this manner just as readily as where he used diseased leaves. He states (p. 15), "From such facts one would conclude that the disease must be inheritable, but experiments do not show this to be so." In regard to this characteristic of mosaic disease Sturgis (1899, pp. 247-8), says that seed from diseased plants do not give rise to "calicoed" plants. "It would seem apparent, therefore, that "calico" is not communicable through the seed. I secured from the seed bed—twenty seedlings showing "calico" and from the same bed, twenty apparently healthy seedlings. These were—set in two parallel rows in the garden—with one exception, all of these forty plants were badly calicoed within six weeks. The exception was one of the originally healthy plants—most of the plants flowered and ripened an abundance of seed. This seed was sown in flats in the greenhouse. Of the hundreds of seedlings—thus raised not a single one showed a sign of "calico" in the flats. Thirty seedlings were transplanted and set in a row in the Station garden—. All of the plants—showed great vigor and remained perfectly healthy. Meantime, from the same lot of seedlings, a dozen were sent to Mr. Ackley, who set them in a warm corner near the barn—. These also failed to show any signs of "calico."

"Tomato mosaic is an inheritable disease in contrast with tobacco mosaic." these are the conclusions of Westerdijk (1910, p. 20). She kept the seed from apparently healthy looking fruit on a diseased plant, separate from that of mottled fruits. She sprouted the seed and the seedlings were transferred to the greenhouse, test plot and garden. Proper checks were used in all cases. All plants grew equally well at first, but in two or three months a noticeable difference was seen. In the field she raised 50 plants, grown from diseased seed; the parent plants having been artificially inoculated. Also 46 were grown from diseased seed from greenhouse plants. Of the latter, 20 originated from mottled

and 26 from apparently normal fruit from diseased plants. All this second generation showed an intensive leaf reduction; the yellow spots appeared entirely inconspicuous. Variegated examples did not occur. The plants grown in the garden showed abnormal appearances all at the same age. Leaf reduction was less noticeable, although leaf apices and side shoots were somewhat abnormally developed. A pronounced case of disease did not occur. In the greenhouse, the plants showed indefinite cases of mosaic disease. One plant out of 27 had strong symptoms of leaf reduction. She states (p. 17), "By the field experiments it has been shown without a doubt that the disease is inheritable. Also here it is shown that the light factor is important in developing the disease."

*Resistance and Selection.*—Hunger (1905) believes through proper selection a resistant strain of tobacco can be obtained. (p. 297). "On page 282 it was shown how diversely plants may develop from Deli seed, even when of the same variety, and I am convinced that it is possible, through proper selection of such seed to isolate and obtain constant physiological strains whose peculiarities would remain fixed within certain limits of temperature." Bouygeres and Perreau (1904) claim to have reduced mosaic disease 98% in a season by selecting seed from a plant which remained healthy among a diseased lot.

#### VARIOUS NAMES FOR SAME DISEASE.

Considerable confusion and dispute exists among European investigators, as to whether Pockenkrankheit, Fleckenkrankheit (Spot disease) and mosaic disease, are the same or different. Mayer (1886) describes the Mosaikkkrankheit, in its second stage by saying that the yellow areas gradually become brown and eventually dry up. These are also the views held by Prillieux and Delacroix (1894); and Marchal, Gontiere and Bouygues (according to Hunger 1905). This stage corresponds to the disease described as Pockenkrankheit by Iwanowski (1892) (b), who noticed it in 1888, and on account of the differences in appearance gave it the distinctive name, "Pockenkrankheit," (Pox Spot). He says (p. 68), "The Mosaic disease is contagious, but such is not the case with Pockenkrankheit. The condition producing Pockenkrankheit is excessive transpiration." He criticises (1902) Beyerinck, Koning and Heintzel for considering Pockenkrankheit and mosaic disease the same trouble. On the other hand, Delacroix (1905) assigns the name "rouille blanche," to a spotting of tobacco caused by a specific bacterium. He says "rouille blanche" must be limited to the so-called Pockenkrankheit, as named by Iwanowski. Westerdijk (1910) states that Pockenkrankheit ("necrobiotische form"), does not occur on the tomato, but that it is very common on tobacco; even more

so than the "yellow-green mosaic" which is scarcely known to many tobacco growers. Sturgis (1899, p. 258) states, "It is evident that in this so-called "spotted disease" of tobacco, we have a disease very similar to, if not identical with, that known in Connecticut as "spotting" and furthermore, that this disease is as distinct from mosaic of foreign tobacco as "spotting" is from "calico." If the statements of the Russian investigators above mentioned are correct (and there is every reason for so regarding them), "spotting" is probably due to excessive transpiration induced by sudden atmospheric changes." In regard to "spotting," in this country, he says (1899, p. 254, "It is a peculiar disease, not very common, not confined to any one locality and not characteristic of any special soil. As I have seen it—it is signalized by the presence on the leaf of small circular spots. These usually occur in the greatest numbers at or near the tips of the leaves, at first—yellow in color—irregular in outline—. The tissue within the border finally dies and becomes almost white, but except in severe cases, it does not break away from the leaf." He goes on to say that microscopic examinations have never shown the presence of fungi or bacteria. "Nothing further, therefore, can be said regarding this trouble, nor would it have been considered worthy of mention were it not for its resemblance to a disease of tobacco which occurs in Europe and Asia." Woods (1902) does not seem to mention this trouble.

There is no serious confusion in this country regarding these troubles; they seem to be distinguishable. According to Sturgis (1900), the "spotting" which may occur at times is not undesirable to a limited extent, as it enhances the value of tobacco. It is sometimes artificially produced by spraying with certain chemicals.

#### CAUSES OF MOSAIC DISEASE.

The causes which have been assigned to this disease are numerous and varied. A great many have been recklessly assigned, as often is the case when some undetermined disease has long resisted the efforts of investigators. According to Hunger (1905) it is still believed by many growers in Europe that "bad intentions" on the part of some one had much to do with its appearance. In Deli it was claimed that the disease appeared where the Coolies had urinated on the plants in the hot-bed, while in other cases laborers were accused of possessing the "warm hand."

Among recent students the cause of mosaic disease is generally considered to be due either (1) To bacterial infection, (2) The Virus theory, (3) A physiological disturbance.

1. *The Bacterial Theory.*—Here a specific organism, a bacterium, is stated to be the cause of mosaic disease. The supporters

of this theory are, Mayer (1886); Iwanowski (1892) (a) (1901, 1903); Prillieux and Delacroix (1894); Marchal (1897); Koning (1899 a, 1900 b); Breda de Haan (1899); Behrens (1896).

Mayer (1886), was perhaps one of the first to suggest bacteria as the cause, saying that the disease is of a bacterial nature. He says, however, that the organism had not been isolated and that nothing is known about its form. Breda de Haan (1899) as quoted by Hunger (1905, p. 262), claims it possible to obtain a bacterium from the plant tissues and grow it in culture. Prillieux and Delacroix (1894) state that a bacillus 0.7 mic. long was associated with grey or yellow spots occurring on tobacco leaves, which they took to be mosaic disease. Marchal (1897), speaks of finding colonies of bacteria which grew in chains and were yellow colored. He claimed that infection occurred in the seed-bed. According to Hunger (1905, pp. 259-60), however, Iwanowski was the first to find bacteria in connection with mosaic disease and certainly his work is the most complete and most convincing that has appeared in support of the bacterial theory. In (1899, p. 253) he reports, "From a poured plate in which one-half drop of mosaic diseased juice was applied, ten transfers from different colonies were made to test tubes, and from each of these, three plants were inoculated. From numbers 6 and 9, two plants showed symptoms of typical mosaic disease within 2 or 3 weeks." In a second preliminary paper (1901, p. 148), he says, "Therefore a specific bacterium is the cause of mosaic disease—." He claims that its discovery is merely a question of proper microtechnique. His final paper (1903) discussed various bacteria obtained from mosaic disease and gives photographs showing them as they occur in host cells. According to him the reason that Beyerinck was not successful in his attempts in isolating bacteria by applying juice to agar tubes, was because it was first filtered, which he says prevented growth. He states (p. 37), "One of the simplest reasons for not having been able to grow this organism from filtered juice is, that the microb is incapable of growing in pure culture and only develops in connection with other bacteria in the soil and in the living plasma of the plant." Such filtered juice, however, will produce the disease. This, he explains, by saying, that the microb forms resting spores. Upon this assumption he believed the microb could be grown only from the vegetative form. He used agar plates and succeeded in obtaining two colonies which produced mosaic disease when reinoculated. He does not mention how or where he made his inoculations and his controls do not appear to be adequate. The percentage of disease produced by his artificial inoculations was small as compared with ordinary juice inoculations; this, he explains as due to a reduction in virulence, as often is the case when bacteria are grown on artificial media.

He describes the bacterium which he used successfully for inoculation purposes, but did not make thorough studies of its habits. It is 0.3 mic. long; in fresh cultures it forms quite long threads or chains. It may liquify gelatin under certain conditions, staining it black. He concludes by saying, that the question of the artificial culture of this microbe of mosaic disease needs further study. Hunger (1905), however, reports that he succeeded at times in obtaining minute bodies which he says might be taken for bacteria. But he says (p. 264), "In fact, I was able to obtain minute bodies at times following out the technique in a few cases even the plasmodium-like bodies. Unfortunately, however, I cannot regard these as bacteria or zoogloa, since it is shown that both of these bodies disappear when phenolchloralhydrate is used in connection with heat, all remaining cell structures remain undisturbed." In a recent article, Allard (1912), believes that Aphids are carriers of mosaic disease in case of tobacco. According to his experiments, he would not place this malady in the category of purely physiological diseases. He says, that facts at hand strongly suggest the presence of a living, active micro-organism.

In order to reach definite conclusions in a pathological problem of this nature, experiments must be conducted on an extensive scale. The organism should be isolated, grown on various media and its cultural characteristics properly recorded. Proper checks with inoculation experiments are absolutely necessary. An experiment without accompanying controls is of little value. The original organism must be reisolated after inoculation and its presence conclusively demonstrated in the host, before its connection with the disease can be considered established. Inasmuch as this has by no means been accomplished, the bacterial theory cannot be considered as more than a working hypothesis.

2. *The Virus Theory*.—The "contagium vivium fluidum" or virus theory seems to be a kind of variation of the bacterial theory. Beyerinck (1898) abandoned the bacterial theory and proposed this in its place. He says (p. 5), "this is not brought about by a microbe, but through a "contagium vivium fluidum." He regards the virus as a soluble substance and not a corpuscular body. It remains inert in dead organic material, but when mixed with the cell plasma, it increases in quantity, but does not lose its individuality, hence the name. He regards the Flecken-krankheit of tobacco as a mild form of the disease, largely confined to the chloroplasts, while in the more intensive forms the protoplast as a whole is involved. His theory is based upon two considerations. (1). The virus must be a liquid and not a corpuscular body, because it diffuses through agar, which is impossible for a corpuscular body. (2). He believes that it must increase in the plant, because a small drop causes numerous

leaves and shoots to become infected. In regard to the first argument of the virus theory, we see it is not quite in accord with our present knowledge of colloidal diffusion; he eliminates a possibility. The second statement is an assumption, rather than a known fact, for the behavior of the injected juice is problematical.

Regarding the amounts of juice required for inoculation he says, (1898, p. 5), "a small drop injected into the plant at the right place will cause numerous leaves and shoots to become infected. If these diseased areas are then crushed and the juice injected into healthy plants they may become diseased." From the fact that pouring juice upon the soil causes the disease to appear first upon the youngest leaves, he concludes that the virus has a definite course in the plant. He applied juice and pieces of diseased tissue to agar plates and allowed the virus to diffuse. He carefully separated the upper and lower strata of such agar and used it for inoculation purposes and produced the disease in each case although the disease appeared more slowly when the lower strata was used. It seems strange that this author did not get a bacterial growth from such plates as Iwanowski did. Lodewijks (1910) hypothesizes a virus in these diseased plants which continually disturb merismatic regions. In normal regions an antiviral is produced which helps to neutralize the virus, like a toxin and an anti-toxin. The formation of this virus and anti-virus is influenced by external conditions; when the former is produced in excess, the plant becomes mosaiced and if the anti-virus is more abundant immunity results. Westerdijk (1910) speaks of a virus in tobacco and tomato, but does not express her opinion as to their nature. She believes that the virus of tobacco is distinct from that of the tomato. She says (1910, p. 19), "There are, therefore, two different infectious substances; they affect only their respective hosts." In her histological studies she excludes organisms as a cause, saying, (p. 8), "No organisms were found, neither in the yellow nor blue-green areas."

(3). *The Physiological Theory*.—Perhaps the most varied, but generally accepted theory is the Physiological one. Some investigators explain this disease as an enzymic trouble, while others simply say that it is of a physiological nature, without mentioning any specific factor or group of factors which can be definitely correlated with it. Sturgis (1899), in his first work on tobacco mosaic states, that artificial injuries or abnormal conditions, whereby the functions of the plant are disturbed, are probable factors in producing this disease. Soil and atmospheric conditions are important agencies according to his views, and he says that mosaic disease is more prevalent in heavy soils. Hunger (1902), believes this disease to be physiological, occurring when the plants are in a weakened condition, predisposed plants

succumbing from the effects of certain outward, injurious influences. In a later paper (1905), he states that mosaic disease is simply due to a disturbance in the metabolism of the host. Meteorological conditions, during the growing season, at least in the case of tobacco, are influential agents and the physical properties of the soil are more important than the chemical. He regards the normal tobacco plant as having mosaic disease in a latent state, or at the least being predisposed towards it, its appearance depending upon external conditions. Westerdijk (1910) says, that mosaic disease is worse in the tropics where light intensity is stronger. She shows that shading tomato plants in the greenhouse has a marked effect in controlling this malady. Heintzel (1900) also believes that this trouble can be explained from the physiological standpoint, but he restricts the cause to abnormal conditions resulting in a localized overproduction of oxidizing enzymes. He states (p. 42), "From various observations I believe, that this disease producing substance in the tobacco plant is an enzyme, or apparently enzymic in nature, which forms or is produced from or by the plant itself under certain conditions." He describes this enzyme by saying that "it is precipitated by alcohol; is soluble in water; loses its properties on boiling; but lowering the temperature even to freezing has no effect upon it; it does not increase outside of the host; salicylic acid interferes with its active properties; it retains its active properties in the dry state as well as in solution; it is diffusible, disturbs cellulose and chlorophyll; at the same time it forms a gas, oxygen." All these properties so closely relate it to an enzyme, that one can call it an enzyme without a doubt." He closes his paper by saying (p. 45), "The enzyme which causes the mosaic disease of tobacco, is therefore, known as an oxidase." Koning (1900) mentions, that he observed a peculiar dark rose color on media, whenever he placed pieces of diseased tissue on agar plates; this being more noticeable than in cases where healthy pieces were used. It appeared to him as though an oxidizing body existed. This seems to harmonize with Woods' (1899, p. 751), results, showing that peroxidases at least, are diffusible. He found that peroxidases would diffuse into agar, if small pieces of *Hibiscus* wood were placed upon such media.

The most detailed and convincing work in support of the enzymic theory, however, has been done by Woods (1899, 1902). He believes as Sturgis (1899) does, that soil conditions are important factors to be considered, (1902, p. 23). "Close clayey soils, packing hard after rains and requiring constant tillage are not favorable to even growth of either the top or the roots of tobacco plants." In the south poorly drained soils are said to favor the development of the disease. He is not of the opinion that a lack of soil nutrients has anything to do with its appearance.



But he states that there is evidence that rapid growth, caused by excessive nitrogenous manure or too high a temperature, is favorable to it. This latter statement seems to correspond with observations made by the writer on the appearance of some cases of tomato mosaic under glass. Woods (1902), does not explain why nitrogenous fertilizers should act in this manner; the plants are really in need of reserve nitrogenous compounds, as will be seen later. He says, however, (p 23), "It is probably connected, however, with the manufacture of reserve nitrogen by the cells and its distribution to the rapidly growing parts." He thinks that tobacco mosaic is especially liable when moist cloudy weather, stimulating rapid growth, is followed by hot, dry weather, checking growth and causing the soil to bake, so that cultivation is apt to injure the root system.

He carried out inoculation experiments along the same lines as other investigators, showing that this disease is infectious. He performed other experiments however, to prove that mosaic disease could be produced at will without employing the juice of diseased or healthy plants. He was able to produce mosaic disease on tomato plants by severally pruning them. Pot-bound tobacco plants were selected and after they had been cut back, (allowing two or three lower leaves to remain), they were submitted to high temperature and copious watering. The rapidly developing shoots became mottled and often distorted. Mosaic disease appeared in plants which were simply punctured with a steril scalped and in other cases where a piece of healthy leaf was inserted. Juice of diseased plants, boiled and double boiled when injected into the terminal bud, or poured around the roots caused the appearance of the disease. Woods (1899, p. 753) says, "It seems plausible that in rapid, poorly nourished growth many of the cells were unable to develop their normal amount of chlorophyll by reason of the excessive development of oxidizing enzymes."

*Oxidizing Enzymes.*—Woods states (1902, p. 23), "The disease is not due to parasites of any kind, but is the result of defective nutrition of the young dividing and rapidly growing cells, due to a lack of elaborated nitrogenous reserve food accompanied by an abnormal increase in activity of oxidizing enzyme in the diseased cells." According to Woods (1902), this excess of oxidases in turn inhibits diastatic activity so that starch accumulates in diseased cells in abnormal quantities. The resulting imperfect translocation may be demonstrated by the application of iodine at different hours during a day. By this means a striking difference between the normal and the abnormal tissue may be demonstrated. Suzuki (1902) arrives at similar conclusions, in the study of his mulberry disease; he confirmed Woods' experiments, showing that it was brought on by excessive pruning and that there was an

overproduction of oxidases in the variegated leaves. He says (1902, p. 277). "The formation of oxidases and peroxidases in abnormal quantities is a peculiar symptom of this disease and at the same time one notices that the translocation of starch and nitrogen compounds is noticeably delayed, so that appreciable quantities of starch are accumulated." He (1902) confirmed Woods' (1899, 1902), experiment on the inhibiting effect of oxidases on diastatic action. Hunger (1903, 1905) and Shibata (1905) were not able, however, to confirm Woods' work and Hunger criticises this theory, believing that Woods worked with impure enzyme solutions and that it was not the oxidase, but rather the tannin which interfered with the diastatic action. Woods (1899, p. 749), however, had shown that diastatic action is hindered even if tannin is removed so that the retardation must be due to the oxidases present. He is not certain that the inhibiting action is as marked during warm weather and under natural conditions. One would naturally expect that such an interference would hinder the production of sugars and proteid compounds. It is on account of of this Woods (1902) believes, that cells of the diseased areas are very poor in reserve nitrogen. Suzuki's (1902) chemical analysis shows this to be the case with the mulberry disease.

Woods (1899, p. 750) finds that "peroxidase is always more than twice as strong in the light colored areas as in the green." In albino spots he found the oxidase twice as strong as in the green areas of the same leaf or in healthy leaves." (p. 753); "It has been suggested by Dr. Loew that partial starvation may cause the increase of these enzymes in a cell, and it has been shown by Brown and Morris, that starvation causes an increase of diastase in the cells of various plants." These enzymes occur throughout the plant according to his statements and when diseased plants disintegrate the enzymes enter the soil and may later be taken up by other plants. Heintzel (1900) and others are also of the opinion that the disease may be disseminated in this way.

Woods (1902) is not able to explain the infectious nature of this disease in accord with the facts, unless the oxidizing enzymes artificially introduced into the plant have the power of evolving these changes. He believes that a zymogen exists for these enzymes. By boiling juice from diseased plants he apparently destroyed the oxidizing enzymes which preliminary tests had shown to be present. After this same juice had been allowed to stand for a day, further tests gave a strong reaction for oxidases. A second boiling after four hours was not followed by a regeneration of the enzymes. He concludes, therefore, that the zymogen exists in the cells in sufficient quantities to regenerate practically the original amount of active enzyme. He believes that as soon as the active enzyme is removed or destroyed, it is regenerated by the zymogen. The protoplasm is not supposed to regulate the

relation between the active and reserve enzyme, for the regeneration occurs in dead cells; no new supply of zymogen is manufactured, neither in the expressed juice nor in the functionless or dead cells.

Although Woods' theory attempted to explain the behavior of these enzymes, his views are not now quite in accord with the rapidly changing ideas concerning this class of enzymes. He does not attempt to explain their mode of action upon inoculation in the host. No statements are made as to the means by which a minute drop of juice injected in the proper place brings about such transformations as are observed in mosaic disease. It is well known that zymogens exist for enzyme processes in which hydrolytic actions occur. Starling (1902) has shown that trypsin of the pancreatic juice is actually secreted as a zymogen, trypsinogen, which lacks proteoclastic power, but possesses other properties similar to those of trypsin itself. The oxidizing enzymes seem to be far more complex and the intimate and intricate mechanism of this group is not so well understood. There seems to be no satisfactory explanation of the increased abundance of oxidizing enzymes in diseased areas of leaves. The methods employed by Woods (1899) for determining the presence of these oxidases were simply colormetric tests, since the reactions accelerated by the juice involve a change in color. Various indicators were used, of which tincture of guaiacum was most satisfactory. He designated those enzymes which gave a reaction directly with guaiacum, as oxidases, those requiring an addition of hydrogen peroxide, peroxidases. This classification is no longer used, see Bayliss (1911, p. 109). Woods' tests were simply qualitative and cannot be depended upon for various reasons as Foa (1908) points out. Guaiac resin for example, assumes a blue color on oxidation, but loses it when the process of oxidation is continued beyond a certain stage. He also gives one to understand that oxidases and peroxidases are not always constant in their mode of action. A certain result in the oxidation of any particular substance gives no ground for generalization as to the catalytic power in general.

Up to the present time no manometric analysis of plants affected with mosaic disease seems to have been made. Such methods have been devised and employed by Mathews (1909) in the Spontaneous Oxidation of Sugars and Bunzel (1912, 1913) on the curly-top of beets. It is obvious that such an analysis would bring out the exact relationships which exists between these enzymes, in healthy and diseased leaves or in any specific areas of such leaves.

#### PREVENTIVE MEASURES.

Various measures have been suggested by scientists and growers for the purpose of controlling or preventing the appearance of mosaic disease. Most of the remedies for tobacco mosaic are

based upon soil treatment or reduction of light intensity. Mayer as early as 1886, showed that renewing soil in the hot-bed gave wonderful results in reducing the disease. By proper liming and shading, Sturgis (1899, 1900), showed that tobacco could be grown practically free from mosaic disease, on soils where calico had been prevalent. Koning (1899) regarded the use of lime and mineral fertilizers as valuable aids to the production of a healthy crop. Loew (1900, p. 25) says, "Some planters entertain the belief that a too extensive use of mineral fertilizers favors the disease and indeed, those fields had the least number of diseased plants which had received chiefly organic manure." The use of new soils for seed-beds and a seed treatment with copper sulphate, is proposed by Gontiere (1900). Eliminating root injury in all ways; preventing too rapid a growth due to using an excess of nitrogenous fertilizer and avoiding improperly drained soils, are Woods' (1902) ideas for combatting the disease. Hunger (1903, 1904, 1905) believes that diseased plants and roots tide the disease over from year to year, and recommends that they should be removed from the fields. He regards the avoidance of all injuries to plants important. Bouygeres and Perreau (1905) advise the elimination of manures. Hinson and Jenkins (1910, p. 10) say, "So far the only known methods of lessening "calico" in the seed-bed, are avoiding the use of tobacco water, as noted before, and the probable good resulting from steam sterilization." Different light intensities and the use of colored lights are possible factors influencing this disease, according to Lodweijks (1911).

The prevention of tomato mosaic under glass is discussed by Westerdijk (1910). She states (pp. 6-7), "The grower can reduce this disease by white-washing the greenhouse as soon as the first signs of yellow spots are noticed." As mentioned before, the writer has observed that over forcing is liable to cause its appearance in the greenhouse.

#### OTHER PLANT DISEASES APPARENTLY OF AN ENZYMIC NATURE.

Besides the work of Woods (1899, 1902), Heintzel (1900) and Hunger (1903) on tobacco mosaic and Suzuki (1902) on the Mulberry disease, mentioned above, there are several more recent investigations which take up certain pathological problems from the standpoint of the enzymic disturbances involved. Pozzi-Escot (1905) assigns various maladies to an over abundance of oxidases. It is believed that a counter action takes place between these and beneficial enzymes which are active in metabolism. Sorauer (1908), in making a study of the leaf curl of potatoes, found that no specific organism was connected with this trouble, but an enzymic disturbance did present itself. In comparing the diseased and healthy tubers, he found great differences in enzymic reactions. Appel and Schlumberger (1911) have considered this

problem from an etiological standpoint. Curly-Top of sugar beets has been an exceedingly baffling disease. Not until (1908) did investigators grasp the situation and the cause was not discovered until (1910). In this year Shaw proved it to be due to an active agent introduced by the bite of the beet leaf hopper. In (1912) Bunzel devised his apparatus for measuring the oxidase content of plant juices quantitatively, and applied it in determining the oxidase content of curly-top of beets in 1913, showing that the leaves of curly-top plants have an oxidase content two or three times that of healthy leaves. During the past year the writer has made a study of an apparently similar disease of the Raspberry, known as Raspberry Yellows or Curl, which although never previously reported, has occurred quite abundantly in Ohio for the last seven years. In addition to these, Peach Yellows, Little Peach, Peach Rosette and other plant diseases have often been regarded as enzymic diseases, but the writer knows of no detailed investigations of the enzymes supposedly concerned.

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## EXPLANATION OF PLATES.

## PLATE VII.

A photograph of leaf showing the mottled effect; the light spots were the yellow areas between the veins. Transmitted light was employed in securing this photograph.

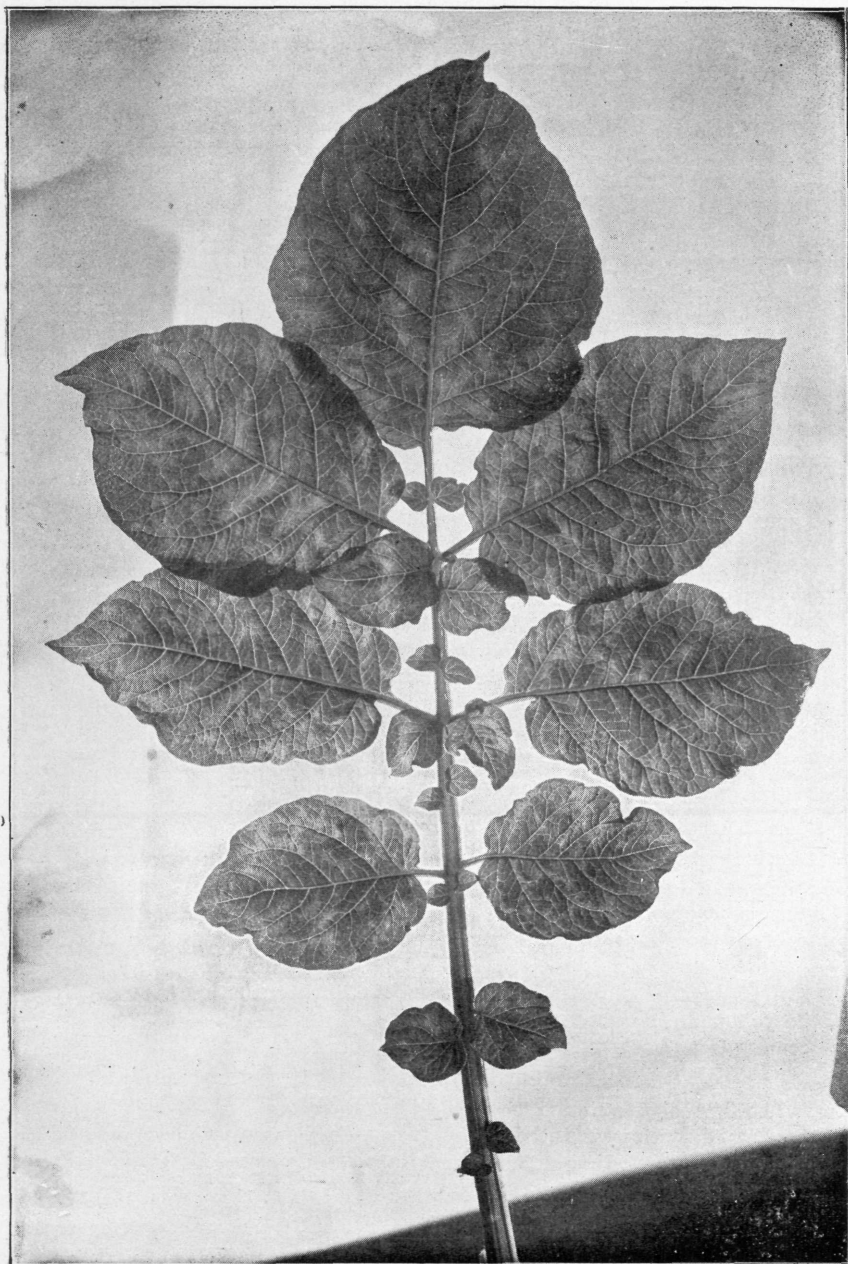
## PLATE VIII.

The figures were drawn with the aid of a camera. A one inch ocular and 4mm. objective were used in each case. The figures have been reduced one-half. Matured tissues of the same age were selected for making the drawings.

Fig. 1. A yellow area showing the cuboidal palisade cells.

Fig. 2. Section from a healthy leaf.

Fig. 3. Section from a green area adjoining a yellow spot.



MELCHERS on "The Mosaic Disease of the Tomato and Related Plants."

